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(54) Title: DIAGNOSTIC TEST DEVICE UTILIZING FILAMENTS CONTAINING REAGENTS

(57) Abstract

The present invention provides a variety of lateral flow test devices for detecting the presence or quantity of an analyte in a test sample utilizing reagent impregnated filaments affixed to a carrier matrix.

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Description

DIAGNOSTIC TEST DEVICE UTILIZING FILAMENTS CONTAINING REAGENTS

Technical Field

The present invention relates generally to diagnostic test devices for the detection of analyte(s) in a test sample wherein the detectable response is indicative of the presence and/or concentration of the analyte(s).

Background of the Invention

Test devices in the form of test strips have become commonplace in the analysis of various types of samples, particularly biological fluids. Test strips designed for detecting clinically significant agents in biological fluids, such as serum and urine, have been advantageous in the diagnosis of disease and pregnancy.

Test strips, such as the pH test paper devices and *in vitro* diagnostic test kit devices, for the detection of various agents have been used for many years in a variety of fields to detect agents such as glucose, protein, and occult blood in biological samples such as urine and blood. Reagent compositions utilized in these devices interact with a constituent analyte(s) in the test sample which are present in liquid samples at concentrations in the millimolar range or above. Unfortunately, many constituent analyte(s) used to identify certain diseases or pregnancy evade detection at this level of sensitivity.

Other test devices utilizing specific binding assay techniques have provided useful analytical methods for determining various organic substances of diagnostic, medical, environmental and industrial importance which appear in liquid test samples below the millimolar concentration range. Specific binding assays are based on the specific interaction between the analyte, *i.e.* the bindable agent

or ligand under determination, and a corresponding binding partner (*i.e.* specific binding reagent). Where the specific binding reagent is an antibody and the analyte is a corresponding hapten or antigen, the assay is known as an immunoassay.

In conventional specific binding assay techniques, a test sample of the liquid to be assayed is combined with various reagent compositions. Such compositions include a labeled conjugate comprising a binding component (*i.e.* specific binding reagent) incorporated with a label. The binding component in the labeled conjugate participates with other constituents, if any, of the reagent composition and with the analyte in the sample under assay. This forms a binding reaction system in which two species, a bound-species and a free-species, of the labeled conjugate are formed. In the bound species, the binding component of the labeled conjugate is bound by a corresponding binding partner, *e.g.* an antibody, whereas in the free-species, the binding component is not bound. The relative amount or proportion of the labeled conjugate that results in the bound-species compared to the free-species is a function of the presence or amount of the analyte to be detected in the sample.

Where the labeled conjugate in the bound-species is essentially indistinguishable in the presence of the labeled conjugate in the free-species by the means used to monitor the label, the bound-species and the free-species must be physically separated in order to complete the assay. This type of assay is referred to as a "heterogeneous assay". Where the bound-species and free-species forms of the labeled conjugate can be distinguished in the presence of the other, the separation step can be avoided, and the assay is said to be "homogeneous".

The earliest developed type of highly sensitive specific binding assay was the radioimmunoassay which utilized a radioactive isotope as the label. Unfortunately, this assay was heterogeneous since the bound- and free-species forms of the labeled conjugate required separation to complete the assay. Because of this inconvenience and difficulty of handling and disposing of

radioactive materials, homogeneous assay systems have been devised using materials other than radioisotopes. These materials included enzymes, bacteriophages, metals and organometallic complexes, coenzymes, enzyme substrates, enzyme activators and inhibitors, cycling reagents, organic and inorganic catalysts, prosthetic groups, chemiluminescent reactants and fluorescent molecules. These homogeneous specific binding systems provide a detectable response, *e.g.* electromagnetic radiation signal such as chemiluminescence, fluorescence emission, or color change related to the presence or amount of the ligand under assay in the liquid sample.

Many commercially available test kits for performing specific binding assays usually comprise a combination of containers holding solutions or rehydratable compositions of the reactants necessary to perform the assay. These methods generally require aliquots of such solutions to be manually or instrumentally dispensed into a reaction vessel with the sample. Unfortunately, if the aliquots are manually dispensed, the assay is dependent on the skill of the technician and if instrumentally dispensed the assay requires the expense and maintenance of the dispensing apparatus.

Several solid phase test devices have been applied to heterogeneous specific binding assays. Commonly used solid phase devises of this type comprise a nonporous surface, such as the interior surface of a test tube or other vessel, to which an antibody is affixed or coated by absorption or covalent coupling. Another solid phase device known as the "gamma-stick" is a test device incorporated with the antibody reagent which is brought into contact with the liquid sample and with the remaining agents of the reaction system, principally the labeled conjugate. After an incubation period, the solid phase device is physically removed from the reaction solution and the label measured either in solution or on the test device.

Similar devices also utilized the antibody reagent entrapped in a matrix such as a gel or paper. Likewise, devices have

been described wherein the antibody reagent is fixed to a matrix held in a flow through column. Such a test device is usually incorporated with less than all the necessary reagent for performing the assay and is merely a means for rendering more convenient the separation step.

Finally, heterogeneous specific binding assay test devices have been described wherein most or all of the necessary reagents are incorporated with the same carrier matrix, and wherein reagent/sample contacts and separation of the free- and bound-phases are accomplished by capillary migration along the carrier matrix. Unfortunately, the heterogeneous specific binding devices described above are generally difficult to manufacture and susceptible to irreproducibility.

Solid phase test devices have also been applied to homogeneous specific binding assays. A solid phase device of this type is simplified to the steps of contacting the device with the sample and measuring the resulting signal, either by visual observation or instrumental means. Reagents would be provided in a solid form, with no need to store, dispense or mix liquid reagents. Such solid state devices would also be more adaptable to automation than previous liquid systems.

Homogeneous specific binding assays have employed a variety of labels, including chemiluminescent labels, enzyme substrate labels and coenzyme labels. In these assays the reagents are incorporated with various carriers, including liquid holding vessels or insoluble, porous, and preferable absorbent, matrices, fleeces, or flocks, gels and the like. Reagents are impregnated into these carriers by a variety of methods resulting in either covalent or non-covalent binding of the reagent to the carrier. In one method a portion of the carrier is immersed in a reagent solution followed by drying while in another method the reagent solution is sprayed onto the carrier and then dried. Unfortunately, impregnation does not provide clear boundaries from which the result of the assay can be easily read. This is because capillary action causes the reagent

solution to migrate within the matrix immediately upon contact and before drying is complete.

Quantitative homogeneous assays involve immersion of a test strip into a test sample and waiting a predetermined time before comparing the strip with a standardized chart. The color chip on the chart which is closest to the color appearing on the test strip during the predetermined time range is indicative of the amount of the agent being assayed present in the test sample. Unfortunately, there are many disadvantages with such a procedure. For example, if the test strip is read too soon or too late an inaccurate result will occur. In addition, such a procedure requires that the technician have good color acuity. Even a slight color blindness would cause inaccuracy. Finally, the results of color comparison between the moistened strip and a standard color chart will vary from individual to individual.

Consequently, a diagnostic test device which utilizes a one-step assay that is rapid, sensitive, has a stable shelf life, contains no radioactive reagent components, does not require the use of an ancillary color chart, provides clearly defined indicator boundaries for accurate determination of assay results, is suitable for use in the home, doctor's office, clinic or hospital, and requires a minimum degree of skill and involvement from the user would be beneficial.

Summary of the Invention

Within one aspect of the present invention, a test device for detecting the presence of an analyte in a test sample is provided comprising, a carrier matrix, wherein the carrier matrix contains a labeled specific binding reagent for the analyte which labeled specific binding reagent is freely mobile within the carrier matrix when in the moist state and a capture filament affixed to the carrier matrix, wherein the capture filament contains an unlabeled specific binding reagent for the same analyte which unlabeled binding reagent is permanently immobilized and is therefore not mobile in the moist state, the relative positioning of the labeled specific binding reagent and the capture filament being such that the test

sample applied to the test device can mobilize the labeled binding reagent contained in the carrier matrix and thereafter permeate to the capture filament where the reagent bound analyte is captured by the capture filament such that the presence of the analyte can be observed.

Within another aspect of the invention a test device for detecting the presence of an analyte in a test sample is provided comprising, a carrier matrix and a reagent filament and a capture filament affixed to the carrier matrix, wherein the reagent filament contains a labeled specific binding reagent for the analyte which labeled specific binding reagent is freely mobile within the carrier matrix when in the moist state and the capture filament contains an unlabeled specific binding reagent for the same analyte which unlabeled binding reagent is permanently immobilized and is therefore not mobile in the moist state, the relative positioning of the reagent filament and the capture filament being such that the test sample applied to the test device can mobilize the labeled binding reagent contained in the reagent filament and thereafter permeate to the capture filament where the presence of reagent bound analyte is captured by the capture filament such that the presence of the analyte can be observed.

Within one embodiment of the invention the test device further comprises a control filament wherein the control filament contains the analyte to be detected permanently bound to the control filament, the relative positioning of the control filament being such that the test sample applied to the test device can mobilize the labeled binding reagent and permeate to the capture filament and thereafter permeate to the control filament where the labeled binding reagent is captured by the control filament such that a control response can be observed.

Within another embodiment of the invention the labeled specific binding reagent for the analyte may be spray dried onto the carrier matrix or may be provided on a filament that is affixed to the

carrier matrix or the carrier matrix may be dipped into a solution containing the labeled specific binding reagent.

The test device may detect a variety of analytes in a test sample, for example, a nucleic acid, a polynucleic acid, a peptide, a polypeptide, a protein, a carbohydrate, a glycoprotein, a steroid or an organic compound. When the analyte to be detected is a protein it may be, for example, an enzyme, an antibody, a receptor, or a receptor ligand. If the protein is an enzyme the enzyme may be, for example, a DNase, an RNase, a protease, a glycosilase, or a carbolipase. If the analyte to be detected is an organic compound the compound may be a metabolic degradation product of a particular drug such as, for example, an amphetamine, a benzodiazepine, a cannabinoid, cocaine, a methamphetamine or an opiate.

The carrier matrix may be composed of a variety of either natural materials, synthetic materials, or a combination of natural and synthetic materials. When the carrier matrix is a natural material it may be, for example, cotton, silk, cellulose or wool. If the carrier matrix is a synthetic material it may be, for example, polypropylene, polyethylene, polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile, or polytetrafluoro-ethylene.

The label of the labeled binding reagent may be a variety of direct or indirect labels, for example, if the label is a direct label, the label may be a gold sol, dye sol, fullerene, a fullerene derivative, latex beads, or erythrocytes. When the label is an indirect label the label may be, for example, an enzyme, a coenzyme, an enzyme modulator, or a quenchable fluorescer. If an enzyme is the indirect label the enzyme may be, for example, a peroxidase, an oxidase, a urease, or a phosphatase.

A variety of specific binding reagents may be utilized in the present invention, for example, the specific binding reagent may be a polyclonal antibody, a monoclonal antibody, a ligand, a peptide, an RNA, or a DNA. When the specific binding reagent is an antibody the

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comprises a porous receiving member linked to the carrier matrix that can act as a reservior to receive a test sample. In one embodiment the hollow casing further comprises a cap preferably constructed of moisture impervious material wherein the cap protects the protruding end of the carrier matrix or the porous receiving member.

In still another aspect of the invention a pregnancy test device is provided comprising a moisture impervious hollow casing containing a carrier matrix which protrudes from the hollow casing and which can act as a reservoir for a urine test sample, and reagent and capture filaments affixed to the carrier matrix, wherein the reagent filament contains a specific anti-hCG antibody labeled with a direct label the labeled specific anti-hCG antibody being freely mobile within the carrier matrix when in the moist state and wherein the capture filament contains an unlabeled specific anti-hCG antibody which is permanently immobilized and is therefore not mobile in the moist state, the labeled and unlabeled anti-hCG antibodies having specificities for different hCG epitopes, the relative positioning of the reagent filament and the capture filament being such that the urine test sample applied to the test device can mobilize the labeled anti-hCG antibody contained in the reagent filament and thereafter permeate to the capture filament where the presence of anti-hCG antibody bound hCG is captured by the capture filament and the moisture impervious hollow casing having at least one aperture through which a detectable response can be observed together with a cap to cover the carrier matrix protruding from the hollow casing.

In another aspect of the invention a fertile period prediction test device comprising a moisture impervious hollow casing containing a carrier matrix which protrudes from the hollow casing and which can act as a reservoir for a urine test sample, and a reagent and capture filaments affixed to the carrier matrix, wherein the reagent filament contains a specific anti-hLH antibody labeled with a direct label, the specific anti-hLH antibody being freely

mobile within the carrier matrix when in the moist state and wherein the capture filament contains an unlabeled specific anti-hLH antibody which is permanently immobilized and is therefore not mobile in the moist state, the labeled and unlabeled anti-hLH antibodies having specificities for different hLH epitopes, the relative positioning of the reagent filament and the capture filament being such that the urine test sample applied to the test device can mobilize the labeled anti-hLH antibody contained in the reagent filament and thereafter permeate to the capture filament where the presence of anti-hLH antibody bound hLH is captured by the capture filament and the moisture impervious hollow casing having at least one aperture through which a detectable response can be observed together with a cap to cover the carrier matrix protruding from the hollow casing.

In another embodiment of the invention a process for the manufacture of these test devices is provided comprising cutting a length of carrier matrix, applying onto the carrier matrix a labeled analyte binding reagent, drying the labeled analyte binding reagent onto the carrier matrix, affixing a length of capture filament onto the carrier matrix, inserting the carrier matrix into a hollow casing having at least one aperture through which a detectable response can be observed such that the carrier matrix protrudes from one end of the hollow casing, and affixing a cap onto the hollow casing to cover the carrier matrix protruding therefrom. A similar process is provided in which the labeled analyte binding reagent is provided utilizing a filament containing the labeled analyte binding reagent. Other processes are provided which incorporate a control filament.

Detailed Description of the Invention

Prior to setting forth the invention, it may be helpful to an understanding thereof to first set forth definitions of certain terms that will be used hereinafter. All references which have been cited below are hereby incorporated by reference in their entirety.

"Analyte" as utilized within the present invention, refers to any agent for which an assay may be developed to detect its presence in a test sample. For example, an analyte may be a nucleic acid, a polynucleic acid, a polypeptide, a protein, a carbohydrate, a glycoprotein, a steroid or an organic compound.

"Carrier matrix" refers to any material or combination of materials that permits lateral flow of a test sample along, through or within the test device and which can be incorporated with specific binding assay reagents. For example, the carrier matrix may be comprised of natural materials such as cotton or wool or comprised of synthetic materials such as polypropylene or ethylene vinylacetate or any combination of natural, synthetic or natural and synthetic materials.

"A labeled or unlabeled specific binding reagent" refers to any agent that binds preferentially to an analyte. It is preferable that the labeled and unlabeled specific binding reagents bind at different locations on the analyte, however, this is not a requirement. For example, the labeled binding reagent may bind to the analyte and the unlabeled binding reagent may selectively bind the analyte bound labeled specific binding reagent.

The term "filament" refers to a thread-like fiber or ribbon, including for example, single fibers or monofilaments, fibers which are combined into threads such as by twisting or into ribbon such as by weaving, monofilaments which are combined into threads and threads which are themselves combined into larger threads or woven into ribbon. The filaments may be composed of a variety of materials, for example, natural materials such as cotton and wool, processed materials such as cellulose or nitrocellulose, or synthetic materials such as polypropylene or ethylene vinylacetate or any combination of natural and synthetic materials.

As noted above, the present invention provides a device for performing specific binding assays. In particular, the present invention provides a carrier matrix having all the reagents necessary to perform a given assay whereby the user has only the task of

bringing the test device into contact with the sample to be tested and observing or measuring the resulting response.

Specific Binding Assays

Reagents for any specific binding assay system may be incorporated in the present invention. In general, homogeneous specific binding assay techniques are based on the interaction between a labeled specific binding reagent and an analyte whereby the presence of the label or a characteristic of the label is different when the labeled specific binding reagent is bound to the analyte as compared to when such analyte is not bound. The affected characteristic of the label may be of any measurable nature such as a chemical or physical quality of the label. Likewise heterogeneous specific binding assay techniques may be incorporated into the present invention. Unlike homogeneous assays the analyte bound specific binding reagent (the bound species) is indistinguishable from the unbound labeled specific binding reagent (the free species) , consequently the bound and unbound species must be physically separated in order to complete the assay. Separation may be accomplished by a variety of methods including, for example, by capillary migration along the carrier matrix.

Binding assays may also incorporate specific binding reagents that bind an analyte in an immunological manner. For example, when the specific binding reagent is an antibody and the analyte is a ligand or hapten of the antibody. Other binding interactions utilized by binding assays include binding interaction between hormones, vitamins, metabolites and pharmaceutical agents and their respective receptors and binding substances.

Where the test sample is being assayed to determine the presence or amount of a particular analyte therein, the reagents for the specific binding assay comprise, a labeled specific binding reagent that binds the analyte composed of, for example, an antibody or receptor or analog thereof coupled to a label, and unlabeled specific binding reagent which interacts with the bound analyte either through binding the analyte or by selectively binding the

complex formed between the analyte and the specific binding reagent, and any ancillary reagents necessary for observing or measuring the signal produced by the labeling substance.

In a competitive specific binding assay a limiting amount of labeled binding reagent bound to the analyte to be detected is combined with the test sample such that the analyte in the sample competes with the analyte bound binding reagent for binding to the unlabeled binding reagent.

In a non-competitive specific binding assay of this type the labeled binding reagent is solubilized in the test sample, wherein any analyte present in the test sample binds to the specific binding reagent, and interacted with the unlabeled binding reagent immobilized on a solid support matrix wherein the analyte bound specific binding reagent is captured. The presence or amount of the analyte in the test sample may be determined by the magnitude of the detectable response from the label.

Either competitive or non-competitive specific binding assay techniques may be easily adapted to a lateral flow system. This system provides a carrier matrix which contains the labeled and unlabeled specific binding reagents. When a test sample is applied to the carrier matrix the test sample migrates along the carrier matrix solubilizing the labeled specific binding reagent. Analyte present in the sample is bound by the labeled specific binding reagent and is carried by the test sample as it migrates to the immobilized unlabeled specific binding reagent. Analyte bound labeled specific binding reagent is captured by the immobilized specific binding reagent and the presence of the analyte is determined by the magnitude of the detectable response from the captured label. Such assay systems are described in U.S. Patent Nos. 4,861,711, 4,094,647, 4,235,601, and 4,361,537.

Several different specific binding assay systems may be utilized in the present invention. The following assay systems are offered by way of illustration and not by way of limitation. The systems are presented according to the nature of the label used.

1. Enzyme Prosthetic Group Labels

In this system, the label is a prosthetic group of an enzyme wherein the ability of a catalytically inactive apoenzyme to combine with the prosthetic group label to form an active enzyme (holoenzyme) is affected by the binding of the labeled specific binding reagent with the analyte. The resulting holoenzyme activity is measured by conventional detection systems. Assay systems of this type are described in U.S. Patent No. 4,238,565. A particularly common prosthetic group-labeled assay scheme utilizes flavin adenine dinucleotide (FAD) as the label and apoglucose oxidase as the apoenzyme. The resulting glucose oxidase activity is measurable by a colorimetric detection system comprising glucose, peroxidase, and an indicator system which produces a color change in response to hydrogen peroxide.

2. Enzyme Substrate Labels

In this system, the label is selected so that the labeled substrate conjugate is a substrate for an enzyme. The ability of the enzyme to act on the substrate label specific binding reagent is affected by the binding of the analyte to the labeled specific binding reagent. Action of the enzyme on the substrate labeled specific binding reagent produces a product that is distinguishable in some feature such as chemical reactivity or photometric character, for example, fluorescence, chemiluminescence or light absorption (color). Assay systems of this type are described in U.S. Patent No. 4,226,978, Anal. Biochem. 77:55 (1977), and Clin. Chem. 23:1402 (1977). A particularly common substrate-labeled assay scheme utilizes a label comprising a galactose bound fluorescent compound. The ability of the enzyme β-galactosidase to cleave this substrate label from the labeled specific binding reagent, yielding a product distinguishable by its fluorescence, is inhibited by the binding of the analyte to the labeled specific binding reagent.

3. Coenzyme Labels

In this system the label is a coenzyme wherein the ability of the coenzyme label to participate in an enzymatic reaction is affected

by the binding of the analyte to the labeled specific binding reagent. The rate of the resulting enzymatic reaction is measurable by conventional detection systems. Assay systems of this type are described in Anal. Biochem. 72:271 (1976), Anal. Biochem. 72:283 (1976) and Anal. Biochem. 76:95 (1976).

4. Enzyme Modulator Labels

In this system the label is an enzyme modulator wherein the ability of the enzyme modulator as an enzyme inhibitor or enzyme stimulator to modulate the activity of an enzyme is affected by the binding of the analyte to the labeled specific binding reagent. The rate of the resulting enzymatic reaction is measurable by conventional detection systems. Assay systems of this type are described in U.S. Patent No. 4,134,792.

5. Enzyme Labels

In this system, the label is an enzyme and the activity of the enzyme label is affected by the binding of the analyte to the labeled specific binding reagent. The resulting enzyme activity is measurable by conventional detection systems. Assay systems of this type are described in U.S. Patent Nos. 3,817,837 and 4,043,872.

6. Quenchable Fluorescent Labels

In this system, the label is a fluorophore whose fluorescence is quenched in some measurable degree when the analyte is bound to the labeled specific binding reagent. The fluorescent label is measured directly, with its fluorescence being the detectable signal. Assay systems of this type are described in U.S. Patent No. 4,160,016 and J. Clin. Path. 30:526 (1977).

7. Chemically-Excited Fluorescent Labels

In this system, the label is a fluorophore, however, the ability of the fluorescer label to be chemically excited to an energy state at which it fluoresces is affected by the binding of the analyte to the labeled specific binding reagent. Chemical excitation of the label may be accomplished by exposure of the fluorescent label to high energy compounds formed *in situ*. Assay systems of this type are described in U.S. Patent No. 4,238,195.

8. Energy Transfer Labels

In this system, the label is one member of an energy transfer donor-acceptor pair and the unlabeled specific binding reagent is conjugated with the other of such pair. When the labeled specific binding reagent is bound to unlabeled specific binding reagent via the analyte the energy expression of the donor component of the pair is altered by transference to the acceptor component. Generally, the donor is a fluorescer and the acceptor is a quencher. The detectable signal is fluorescence and can be measured directly, however, other detection systems may be utilized. Assay systems of this type are described in U.S. Patent Nos. 3,996,345, 4,174,384, and 4,199,559.

9. Direct Labels

In this system, the label may be a variety of visible agents such as dyes, colored latex beads, or erythrocytes wherein a detectable signal is directly affected by the presence or absence of the analyte in the test sample. A particularly common direct label scheme utilizes a colored latex bead as the label. As analyte bound labeled specific binding reagent is captured by the unlabeled specific binding reagent a detectable response develops as the colored latex beads are aggregated into a localized area. Assay systems of this type are described in U.S. Patent No. 4,943,522.

10. Other Labels

As previously stated several different specific binding assay systems may be utilized in the present invention including, for example, nonenzymatic catalysts such as electron transfer agents (*see* U.S. Patent No. 4,160,645), nonchemiluminescers, and labeled liposomes particles (*see* U.S. Patent No. 4,193,983).

Coupling of the label to the specific binding reagent may be performed by a variety of methods known in the art.

Analyte

The present assay system may be applied to the detection of any analyte for which there is a specific binding reagent *e.g.* antigens and antibodies thereto, haptens and antibodies thereto, or ligands and receptors thereto. The analyte may be a variety of agents, for

example, the analyte may be a nucleic acid, polynucleic acid, peptide, polypeptide, protein, carbohydrate, glycoprotein, steroid, nucleic acid, a polynucleic acid, or other organic molecule for which a specific binding reagent exists. Generally, the analyte is an immunologically active polypeptide or protein of molecular weight between 1,000 and 10,000,000, such as an antibody, antigenic polypeptide or protein, or a hapten of molecular weight between 100 and 5,000.

Representative polypeptide analytes include, for example, angiotensin I and II, C-peptide, oxytoxin, vasopressin, neurophysin, gastrin, secretin, bradykinin, and glucogon.

Representative protein analytes include, for example, classes of protamines, mucoproteins, glycoproteins, globulins, albumins, scleroproteins, phosphoproteins, histones, lipoproteins, chromoproteins, and nucleoproteins. Examples of specific proteins include for example prealbumin, α1-lipoprotein, α, β and γ interferon, the interleukins, human serum albumin, α1-glycoprotein, transcortin, thyroxine binding globulin, haptoglobulin, hemoglobin, myoglobin, ceruloplasmin, α2-lipoprotein, α2-macroglobulin, β-lipoprotein, erythropoietin, transferrin, homopexin, fibrinogen, the immunoglobulins such as IgG, IgM, IgA, IgD and IgE, and their fragments, *e.g.* F_C and F_{ab}, complement factors, prolactin, blood clotting factors such as fibrinogen, and thrombin, insulin, melanotropin, somatotropin, thyrotropin, follicle stimulating hormone, leutinizing hormone, gonadotropin, thyroid stimulating hormone, placental lactogen, intrinsic factor, transcobalamin, serum enzymes such as phosphokinases, alkaline phosphatase, lactic dehydrogenase, amylase, lipase, phosphatases, cholinesterase, glutamic oxaloacetic transaminase, glutamic pyruvic transaminase, and uopepsin, endorphins, protamine, tissue antigens, bacterial antigens, and viral antigens.

Representative hapten analytes include, for example, the general classes of hormones, vitamins, drugs, and metabolites. Haptenic hormones include, for example, thyroxine and triiodothyronine. Heptanic vitamins include, for example, vitamins A,

B, *e.g.* B_{12}, C, D, E and K, folic acid, and thiamine. Haptenic drugs include, for example, antibiotics such as aminoglycosides, *e.g.*, gentamicin, tobramycin, amikacin, sisomicin, kanamycin, and netilimicin. penicillin, tetracycline, terramycin, chloromycetin, and actinomycetine; nucleoside and nucleotides such as adenosine diphosphate (ADP), adenine triphosphate (ATP), flavin mononucleotide (FMN), nicotinamide adenine dinucleotide (NAD), and its phosphate derivative (NADP), thymidine, guanosine, and adenosine; prostaglandins; steroids such as the estrogens, *e.g.*, estriol and estradiol, sterogens, androgens, digoxin, digitoxin, and adrenocortical steriods; and others such as phenobarbital, phenytoin, primidone, ethosuximide, carbamazepine, valproate, theophilline, caffeine, propranolol, procainamide, quinidine, amitryptiline, cortisol, desipramine, disopyramide, doxepine, doxorubicin, nortryptiline, methotrexate, imipramine, lidocaine, N-acetyl-procainamide, the amphetamines, the catecholamines, and the antihistamines.

The liquid medium to be assay may be a naturally occurring or may be artificially formed liquid suspected of containing the analyte. Generally, the liquid is a biological fluid or a dilution thereof. Biological fluids that may be assayed include, for example, serum, plasma, urine, saliva, amniotic fluid, or cerebrospinal fluid.

Carrier Matrix

The carrier matrix of the present invention may be constructed of a variety of natural or synthetic materials and is therefore intended as being broad in context. It may be mono- or multi-phasic, comprising one or more materials or mediums of similar or different absorptive or other characteristics. It may further be hydrophobic or hydrophilic, or bibulous or nonporous in character. In its most efficient embodiment the carrier matrix is tailored to suit the characteristics of the particular specific binding assay system to be employed. Carrier matrixes may be comprised of felt, porous ceramic strips, woven glass fibers, or matted glass fibers (*see* U.S. Patent No. 3,846,247), wood sticks, cloth, sponge material, or argillaceous substances (*see* U.S. Patent No. 3,552,928), synthetic resin fleeces and

glass fiber felts (*see* British Patent No. 1,369,139), polyamide fiber (*see* French Patent No. 2,170,397), polypropylene, polyethylene (preferably of high molecular weight), polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile, and polytetrafluoro-ethylene (*see* European Patent Application 291,194), polyvinyl chloride, polyvinylacetate, copolymers of vinyl acetate and vinyl chloride, polycarbonate, and polystyrene. All such carrier matrix concepts as well as a variety of others may be utilized by the present invention. Preferably the carrier matix is made of porex (Porex Technologies Corporation of Fairburn, Georgia).

The porosity of the carrier matrix may be unidirectional (*i.e.* with pores or fibers that run wholly or predominantly parallel to the axis of the member), multidirectional, or omnidirectional. The carrier matrix may be pretreated with surfactant materials such anionic or non-ionic surfactants to enhance the uptake, extent and range of wetting and lateral flow of a test sample within the matrix. Alternatively, the carrier matrix may be pretreated with a glazing material in the region to which the labeled reagent is to be applied. Glazing may be achieved for example by depositing an aqueous sugar (e.g. sucrose or lactose) or cellulose solution on the carrier matrix at the relevant portion, and drying. The labeled specific binding reagent can then be applied to the glazed portion. It may also be desirable to treat the carrier matrix with an agent to block any remaining binding sites after application of the specific binding reagents. Blocking may be achieved by treatment with for example, protein (e.g. bovine serum albumin or milk protein), or with polyvinylalcohol or ethanolamine, or any combination of these agents. It may also be desirable to include agents that render inactive or nonactive materials which are or may be potentially deleterious to the assay analysis. Preferably, the carrier matrix comprises a porous material that can be saturated with a test sample quickly, remains robust when moist, and which permits the test sample to permeate freely.

It is preferable that the flow rate of an aqueous sample through the carrier matrix should be such that in the untreated

material, aqueous liquid migrates approximately 1cm in not more than about 2 minutes but slower flow rates may be used if desired. It may also be desirable that the carrier matrix comprise reflective pigments, such as for example, titanium dioxide or barium sulfate to enhance signal detection by reflection radiometry, *e.g.* reflection photometry or other similar technique.

Carrier matrixes may be opaque or transparent. If transparent it is preferable that the carrier matrix materials be capable of transmitting electromagnetic radiation in a wavelength within the region between 200 nanometers (nm) and 900 nm. The matrix need not transmit over this entire region although for fluorometric detection of analytical results through the matrix it is desirable for the matrix to transmit over a wider band or alternatively, to transmit at the absorption and emission spectra of the fluorescent materials used for detection. It may also be desirable to have a carrier matrix that transmits one or more narrow wavelength bands and is opaque to adjacent wavelength bands. This could be accomplished, for example, by impregnating or coating the support with one or more colorants having suitable absorptive characteristics.

The labeled analyte specific binding reagent may be applied directly to the carrier matrix by a variety of methods including, dipping a portion of the carrier matrix into a solution containing the labeled analyte specific binding reagent or by spraying this solution onto the carrier matrix. The matrix is then dried or lyophilized. Alternatively, the labeled analyte specific binding reagent may be incorporated into the test device by affixing a filament containing the labeled analyte specific binding reagent.

Preferably the carrier matrix is contained within a moisture impervious hollow casing or housing having at least one open end through which the carrier matrix extends for receiving the test sample. The hollow casing may be translucent or opaque. If opaque, the hollow casing would further comprise at least one aperture through which a detectable response may be observed. A second aperture may be provided for observation of a detectable control

filament response to indicate whether the assay procedure has been completed. Preferably the aperture of the hollow casing forms a translucent convex lens that magnified the capture and/or control filaments when the hollow casing is made of translucent material. If the hollow casing is opaque it is preferable that the aperture contain a translucent convex lens to magnify the capture and/or control filaments. The hollow casing may be constructed in a variety of shapes. One preferable shape is an elongated tube shape on which may be provided a variety of designs. Preferably the hollow casing has a figure of a pregnant human female on the outer surface wherein the translucent convex lens forms the torso of the pregnant human female. Preferably the hollow casing is provided with a removable cap or shroud which protects the carrier matrix protruding therefrom during storage and before use. If desired the cap or shroud may be replaced over the protruding carrier matrix after application of the test sample while the assay is being performed.

The carrier matrix may further comprise a porous receiving member linked to one end to which the liquid sample can be applied and from which the sample can permeate onto the carrier matrix. Preferably the carrier matrix is contained within a hollow casing or housing and the porous receiving member, with which the carrier matrix is linked, extends out of the housing and can act as a means for permitting a test sample to enter the housing and permeate the carrier matrix. Preferably a removable cap or shroud is provided which can protect the protruding porous receiving member during storage and before use. The porous recieving member may be made of any bibulous, porous, or fibrous material capable of absorbing liquid rapidly, for example, porous plastic materials such as polypropylene, polyethylene (preferably of high molecular weight), polyvinylidene flouride, ethylene vinylacetate, acrylonitrile and polytetrafluoro-ethylene.

Filaments

Filaments as provided in the present invention may comprise any natural, synthetic, or combination of natural and synthetic material which would lend itself to being formed into filaments. Natural materials include, for example, cotton, wool, hemp, kapok, soy kestin, silk, paper and zein. Examples of synthetic polymers include regenerated cellulose, polyacrylates, polyolefins, (such as polyethylene and polypropylene) and polyamides (such as nylon).

The reactants of the specific binding assay may be incorporated with the filaments in various ways. For example, a filament comprising many twisted fibers, such as cotton, can be drawn through a bath containing the reagent, thereby saturating the fibers and subsequently dried. This leaves the reagent as a residue within the filament interstices. Another method is to bond the reagent to the surface of the filament. Thus, hydrogen bonding, covalent bonding, hydrophilic interactions, or hydrophobic interactions between polymer filament and reagent may be utilized. In the case of extruded filaments such as polyolefins, the reagent system can be blended with the polymer in a melt prior to melt extrusion. The techniques for incorporating filaments with reagents depend upon the mutual properties of the filament and reagent which are determinable by a person skilled in the art.

By utilizing filaments impregnated with reagents test devices may be constructed which detect the presence of more than one analyte in a test sample. For example, the ratio of the levels of glycated hemoglobin (HbA) to unglycated (HbAo) or total hemoglobin (Hb) can aid in the management of diabetes. Similarly the ratio of levels of apolipoproteins A_1 and B can be indicative of susceptibility to coronary heart disease. Additionally, it is possible to configure a test device to measure two steriods simultaneously such as E-3-G and P-3-G or the presence of viral antigens such as hepatitis associated antigens HBsAg, HBcAg or HBeAg and human immunodeficiency virus antigens such as gp160. By way of example, a dual analyte test for apolipoproteins A_1 and B may be prepared by affixing to the carrier matrix two capture filaments and two reagent

filament(s). One capture filament comprises an immobilized specific binding reagent for apolipoprotein A_1 (*e.g.*, an antibody specific for apolipoprotein A_1) and the second capture filament comprises an immobilized specific binding reagent for apolipoprotein B (*e.g.*, an antibody specific for apolipoprotein B). Both filaments are affixed to the carrier matrix spacially separated from each other and further separated from the reagent filament(s). Each reagent filament contains a single labeled specific binding reagent (either an apolipoprotein A_1 or apolipoprotein B specific binding reagent). The labeled reagents become mobile and migrate with the solvent flow when an aqueous test sample is applied. While migrating toward the capture filaments the labeled specific binding reagents for apolipoprotein A_1 and B bind specifically and preferentially to apolipoprotein A_1 and B respectively present in the test sample. On reaching the capture filaments the analyte bound specific binding reagents are captured by their corresponding immobilized specific binding reagents. In particular, apolipoprotein A_1 bound to labeled apolipoprotein A_1 specific binding reagent is captured by apolipoprotein A_1 immobilized unlabeled specific binding reagent. Correspondingly, apolipoprotein B bound to labeled apolipoprotein B specific binding reagent is captured by apolipoprotein B immobilized unlabeled specific binding reagent. An accumulation of each of the direct labels may occur at both or either capture filament to a lesser or greater extent dependent upon the presence of each apolipoprotein in the test sample. The result is a signal that may be visibly observed.

In a similar assay for the presence of two analytes in one test sample the labels of the labeled specific binding reagents may produce different colored visible signals. For example, in the assay described above the accumulation of analyte bound apolipoprotein A_1 labeled specific binding reagent may result in a visible red color while the accumulation of analyte bound apolipoprotein B labeled specific binding reagent may result in a visible green color (such as when the labels are red and green colored latex beads).

In another variation of the above assay a single reagent filament may be used in which both specific binding reagents are incorporated into the filament. For example, such a filament may be produced by drawing the filament through a cocktail comprised of apolipoprotein A_1 and B, saturating the fibers which is then subsequently dried.

In yet another variation of the invention a plurality of capture filaments for a single analyte may be utilized wherein each filament designates a known quantity of analyte present in the test sample. Consequently, the amount of analyte present in the test sample would be based on the representative analyte concentration that each filament has the capacity to detect times the number of filaments which develop a detectable response. The capacity of a filament may be determined by a variety of methods known in the art. For example, filaments from a single lot may be incorporated into the test device of the present invention and subjected to a series of known concentrations of the analyte to be detected. The number of threads which develop under each concentration of analyte may be used to determine the relative concentration of analyte in an unknown test sample.

If present, a control filament may be incorporated into the test device to convey an unrelated signal to the user that the device has functioned correctly. For example, the control filament may be incorporated with a specific binding reagent that will bind to the labeled specific binding reagent using methods previously described. In particular, if the labeled specific binding reagent were a mouse monoclonal antibody directed against the analyte to be detected, the specific binding reagent of the control filament could be an anti-mouse antibody. Alternatively, an anhydrous agent could be incorporated into the control filament such that when the filament becomes moist color is produced or a color change results. For example, anhydrous copper sulfate may be used which turns blue when moistened by a test sample. As a further alternative, the control filament could contain immobilized analyte which will react

with excess labeled specific binding reagent. Since the purpose of the control filament is to indicate to the user that the test has functioned correctly the control filament should be located downstream from the reagent and capture filaments. A positive control indicator therefore tells the user that the sample has permeated the required distance though the test device.

Detectable Response

Specific binding assays may be readily adapted to provide a detectable response, such as a color change, chemiluminescence, or fluorescence related to the presence or amount of the analyte in the test sample.

The term "detectable response" and similar terms as used herein, refer to the detectable manifestation of the presence of such species. Examples include electromagnetic radiation signals such as fluorescence, phosphorescence, chemiluminescence, and change in light absorption, or reflectance in the visible spectrum thereby producing a visible color change, a change in light absorption or reflectance outside the visible range such as the ultraviolet and infrared. As will be apparent to those skilled in the art of immunoassays, the phrase "detectable response" as used herein, is intended in its broadest sense. In addition to electromagnetic radiation signals, the term "detectable response" is also meant to include any observable change in system parameter, such as change in appearance of a reactant, observable precipitation of any component in the test sample such as agglutination, aggregation of reactants, or a change in any other parameter, whether it be in the immunoassay system or a test sample. Other detectable responses include electrochemical responses and colorimetric responses. Moreover, the detectable response is one which can be observed through the senses directly or by use of ancillary detection means, such as a spectrophotometer, spectrofluorometer, pH meter and other sensing means.

After the analytic result is obtained as a detectable change it may be measured by passing the test element through a zone in

which a suitable apparatus for reflection, transmission, or fluorescence photometry is provided. Preferably the test device results may be read directly without the assistance of an apparatus.

The following examples are offered by way of illustration and not by way of limitation.

EXAMPLES

Example 1

PREPARATION OF LABELS

A. GOLD SOL PREPARATION

Gold sols may be prepared for use in immunoassays from commercially available colloidal gold, and an antibody preparation such as anti-alpha human chronic gonadotrophin (hCG). Colloidal gold G20 (20nm particle size, Janssen Life Sciences Products) is adjusted to pH 7 with 0.22μ filtered 0.1M K_2CO_3, and 20 mls is added to a clean glass beaker. Two hundred microliters of anti-alpha hCG antibody, prepared in 2mM borax buffer pH 9 at 1 mg/ml, and 0.22μ filtered, is added to the gold sol, and the mixture is stirred continuously for two minutes. Approximately 0.1M K_2CO_3 is used to adjust the antibody gold sol mixture to pH 9, and 2mls of 10% (w/v) bovine serum albumin (BSA) is added.

The antibody-gold conjugate is purified in a series of three centrifugation steps at 12000g for 30 minutes at 4°C with only the loose part of the pellet being resuspended for further use. The final pellet is resuspended in 1% (w/v) BSA in 20mM Tris, 150mM NaCl, pH 8.2

B. DYE SOL PREPARATION

Dye sols may be prepared from commercially available hydrophobic dyestuffs such as Foron Blue SRP (Sandoz) and Resolin Blue BBLS (Bayer). Approximately 50grms of dye is dispersed in 1L distilled water by mixing for 2-3 minutes. Fractionation of the dye dispersion is performed by an initial centrifugation step at 1500g for 10 minutes at room temperature to remove larger sol particles with the supernatant suspension being retained for further centrifugation.

The suspension is centrifuged at 3000g for 10 minutes at room temperature, and resuspending the pellet in 500mls of distilled

water. This procedure is repeated three additional times with the final pellet being resuspended in 100mls of distilled water.

The spectra of dye sols prepared in this example may be measured at lambda max. of approximately 657nm for Foron Blue and 690nm for Resolin Blue. The absorbance at lambda max., for 1cm path length is used as a measure of the dye sol concentration.

Anti-alpha hCG antibody or anti-LH antibody is prepared in phosphate buffered saline pH 7.4 at 2mg/ml. A reaction mixture is prepared which contains 100µL antibody solution, 2mls dye sol, 2mls 0.1 phosphate buffer pH 5.8 and 15.9mls distilled water. After gentle mixing, the preparation is left for 15 minutes at room temperature. Excess binding sites may be blocked by the addition of 4 mls of 150mg/ml BSA in 5mM NaCl pH 7.4 to the reaction mixture and after 15 minutes incubation at room temperature, the solution is centrifuged at 3000g for 10 minutes, and the pellet resuspended in 10mls of 0.25% (w/v) dextran/0.5%(w/v) lactose in 0.04M phosphate buffer. This antibody-dye sol conjugate may be stored in lyophilized form.

C. COLORED PARTICLES PREPARATION

Latex particles for use in immunoassays are available commercially. Approximately, 0.5ml (12.5mg solids) of polystyrene beads of about 0.3µ diameter (Polymer Laboratories) is diluted with 1ml of 0.1M borate buffer pH 8.5 in an vial. These particles are washed four times in borate buffer, each wash consisting of centrifugation for three minutes at 13000rpm in a microcentrifuge at room temperature. The final pellet is resuspended in 1M borate buffer, mixed with 300µg of anti-alpha hCG antibody, and the suspension is gently rotated for 16-20 hours at room temperature. The antibody-latex suspension is centrifuged for 5 minutes at 13000rpm, the pellet is resuspended in 1.5mls borate buffer containing 0.5mg BSA. Following rotation for 30 minutes at room temperature the suspension is washed three times in 5 mg/ml BSA in phosphate buffered saline pH 7.4 by centrifugation at 13000 rpm

for 5 minutes. The pellet is resuspended in 5mg/ml BSA/5% (w/v) glycerol in phosphate buffered saline pH 7.2 and stored at 4°C.

Example 2

PREPARATION FILAMENTS

A. PREPARATION REAGENT FILAMENTS

White mercerized, cotton thread (No. 40/3) is impregnated by dipping into a bath containing either the gold-sol labeled anti-alpha hCG antibody, dye-sol labeled anti-alpha hCG antibody, or dye-sol labeled anti-LH antibody prepared in Example 1 above. The thread is passed through the bath at a speed of 1 meter/minute and dried at 60°C.

B. PREPARATION OF CAPTURE AND CONTROL FILAMENTS

The method for coupling an unlabeled specific binding reagent to a filament will depend on the type of filament and nature of the specific binding reagent. A polysaccharide matrix filament is oxidized by mild treatment with sodium metaperiodate. At pH 8.5 to 9.5 the horseradish peroxidase label anti-LH antibody is coupled to the filament. This linkage results through the formation of a Schiff base. After washing with buffer any remaining active sites on the filament are blocked by reduction with sodium borohydride. The filament is then washed and dried to increase stability and immunoreactivity of the antibody.

Example 3

IMPREGNATION OF CARRIER MATRIX WITH UNLABELED SPECIFIC BINDING REAGENT

A 2mg/ml solution is prepared of an anti-beta hCG antibody ($K_{affinity} = 1 \times 10^9$) in phosphate buffered saline pH 7.4 which is suitable for immunoassay of hCG using a second labeled anti-hCG

antibody in a sandwich format. This solution is deposited by means of a microprocessor controlled microsyringe, which delivers precise volumes of reagent through a nozzle, preferably 0.2 to 0.3mm diameter. After being applied the material is allowed to dry for 1 hour at room temperature.

Example 4

hCG LATERAL FLOW SPECIFIC BINDING ASSAY

A piece of Porex (Porex Technologies Corporation, Fairburn, Georgia) carrier matrix material is cut from roll stock to give a dimension of 8cm in width and 30cm in length. Grooves are cut perpendicular to the axis of the matrix. Reagent filaments prepared in Example 3 above are inlayed into the grooves of the matrix such that the gold-sol anti-hCG antibody conjugate is closest to the test sample receiving end, the unlabeled anti-hCG antibody capture filament is affixed downstream from the gold-sol anti-hCG antibody conjugate, and the analyte bound control filament is affixed further downstream from the test sample receiving end. The filament bound carrier matrix is inserted into and secured in a cylinder made of plastic such that the carrier matrix protrudes from one end and the capture and control filaments may be observed through apertures in the cylinder. A urine test sample is applied to the protruding end of the carrier matrix directly from the urine stream of by dipping into a receptacle containing urine. After approximately five minutes or when the control filament changes color the test is complete. If hCG is present in the urine test sample a visible color change in the capture filament can be observed. If no color change is detected the urine test sample does not contain detectable amounts of hCG.

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention.

Accordingly, the invention is not limited except as by the appended claims.

Claims

We claim:

1. A test device for detecting the presence of an analyte in a test sample comprising, a carrier matrix, wherein the carrier matrix contains a labeled specific binding reagent for the analyte which labeled specific binding reagent is freely mobile within the carrier matrix when in the moist state and a capture filament affixed to the carrier matrix, wherein the capture filament contains an unlabeled specific binding reagent for the same analyte which unlabeled binding reagent is permanently immobilized and is therefore not mobile in the moist state, the relative positioning of the labeled specific binding reagent and the capture filament being such that the test sample applied to the test device can mobilize the labeled binding reagent contained in the carrier matrix and thereafter permeate to the capture filament where the reagent bound analyte is captured by the capture filament such that the presence of the analyte can be observed.

2. A test device for detecting the presence of an analyte in a test sample comprising, a carrier matrix and a reagent filament and a capture filament affixed to the carrier matrix, wherein the reagent filament contains a labeled specific binding reagent for the analyte which labeled specific binding reagent is freely mobile within the carrier matrix when in the moist state and wherein the capture filament contains an unlabeled specific binding reagent for the same analyte which unlabeled binding reagent is permanently immobilized and is therefore not mobile in the moist state, the relative positioning of the reagent filament and the capture filament being such that the test sample applied to the test device can mobilize the labeled

binding reagent contained in the reagent filament and thereafter permeate to the capture filament where the presence of reagent bound analyte is captured by the capture filament such that the presence of the analyte can be observed.

3. The test device according to any of claims 1 or 2 further comprising a control filament wherein the control filament contains the analyte to be detected permanently bound to the control filament, the relative positioning of the control filament being such that the test sample applied to the test device can mobilize the labeled binding reagent and permeate to the capture filament and thereafter permeate to the control filament where the labeled binding reagent is captured by the control filament such that a control response can be observed.

4. The test device according to claim 1 wherein the labeled specific binding reagent for the analyte is applied to the carrier matrix by spray drying, by dipping into a solution containing the labeled specific binding reagent or by affixing a filament containing the labeled specific binding reagent.

5. The test device according to any of claims 1 or 2 wherein the analyte is selected from the group consisting of a nucleic acid, a polynucleic acid, a peptide, a polypeptide, a protein, a carbohydrate, a glycoprotein, a steroid and an organic compound.

6. The test device according to claim 5 wherein the analyte is a protein selected from the group consisting of an enzyme, an antibody, a receptor, and a receptor ligand.

7. The test device according to claim 6 wherein the enzyme analyte is selected from the group consisting of DNase, RNase, protease, glycosilase, or carbolipase.

8. The test device according to claim 5 wherein the organic compound analyte is selected from the group consisting of a metabolic degradation product of an amphetamine, a benzodiazepine, a cannabinoid, cocaine, a methamphetamine or an opiate.

9. The test device according to claim 1 wherein the carrier matrix is composed of materials selected form the group consisiting of natural materials, synthetic materials and a combination of natural and synthetic materials.

10. The test device according to claim 9 wherein the carrier matrix is composed of a natural material selected from the group consisting of cotton, silk, cellulose, and wool.

11. The test device according to claim 9 wherein the carrier matrix is composed of a synthetic material selected from the group consisting of polypropylene, polyethylene, polyvinylidene fluoride, ethylene vinylacetate, acrylonitrile, and polytetrafluoro-ethylene.

12. The test device according to any of claims 1 or 2 wherein the label of the labeled specific binding reagent is a direct label or an indirect label.

13. The test device according to claim 12 wherein the label is a direct label selected from the group consisting of gold sols, dye sols, fullerene, fullerene derivatives, latex beads, and erythrocytes.

14. The test device according to claim 12 wherein the label is an enzyme indirect label selected from the group consisting of peroxidase, oxidase, urease, and phosphatase.

15. The test device according to any of claims 1 or 2 wherein the label of the labeled specific binding reagent is selected from the group consisting of a coenzyme, enzyme modulator, a quenchable fluorescer.

16. The test device according to any of claims 1 or 2 wherein the specific binding reagent is selected from the group consisting of a polyclonal antibody, a monoclonal antibody, a ligand, a peptide, an RNA, and a DNA.

17. The test device according to any of claims 1 or 2 wherein the specific binding reagent is an antibody directed against the proteins selected from the group consisting of hCG, IGFBP-1, cardiac proteins, cancer cell markers or bone loss markers.

18. The test device according to claim 2 wherein the reagent filament comprises different labeled specific binding reagents for more than one analyte to be tested.

19. The test device according to claim 2 wherein the reagent filament comprises a plurality of filaments each filament comprising a labeled specific binding reagent for a different analyte to be tested.

20. The test device according to any of claims 1 or 2 wherein there are a plurality of capture filaments.

21. The test device according to claim 20 wherein each filament of the plurality of capture filaments comprise a specific concentration of a specific binding reagent for an analyte to be tested.

22. The test device according to claim 20 wherein each filament of the plurality of capture filaments comprise a specific binding reagent for a different analyte to be tested.

23. The test device according to any of claims 1 or 2 further comprising a hollow casing which contains the carrier matrix, wherein the hollow casing is constructed of moisture impervious material and has at least one open end such that a test sample can be applied to the carrier matrix.

24. The test device according to claim 23 wherein the hollow casing is constructed of moisture impervious material having at least one aperture through which a detectable response may be observed.

25. The test device according to claim 24 wherein the hollow casing is constructed of moisture impervious material having a first and a second aperture, wherein the first aperature acts to receive a test sample and the second aperature permits a detectable response to be observed.

26. The test device according to claim 24 wherein the aperture of the hollow casing is a translucent convex lens that magnifies the capture filament.

27. The test device according to claim 26 wherein the hollow casing comprises a figure of a pregnant human female on the surface and the translucent convex lens aperture forms the torso of the figure.

28. The test device according to claim 23 wherein the carrier matrix protrudes from the hollow casing and can act as a reservoir to receive a test sample.

29. The test device according to any of claims 1 or 2 further comprising a porous receiving member linked to the carrier matrix that can act as a reservior to receive a test sample.

30. The test device according to claim 23 further comprising a cap constructed of moisture impervious material wherein the cap protects the open end of the carrier matrix.

31. A pregnancy test device comprising a moisture impervious hollow casing containing a carrier matrix which protrudes from the hollow casing and which can act as a reservoir for a urine test sample, and reagent and capture filaments affixed to the carrier matrix, wherein the reagent filament contains a specific anti-hCG antibody labeled with a direct label the labeled specific anti-hCG antibody being freely mobile within the carrier matrix when in the moist state and wherein the capture filament contains an unlabeled specific anti-hCG antibody which is permanently immobilized and is therefore not mobile in the moist state, the labeled and unlabeled anti-hCG antibodies having specificities for different hCG epitopes, the relative positioning of the reagent filament and the capture filament being such that the urine test sample applied to the test device can mobilize the labeled anti-hCG antibody contained in the reagent filament and thereafter permeate to the capture filament where the presence of anti-hCG antibody bound hCG is captured by the capture filament and the moisture impervious hollow casing having at least one aperture through which a detectable response can be observed together with a cap to cover the carrier matrix protruding from the hollow casing.

32. A fertile period prediction test device comprising a moisture impervious hollow casing containing a carrier matrix which protrudes from the hollow casing and which can act as a reservoir for a urine test sample, and a reagent and capture filaments affixed to the carrier matrix, wherein the reagent filament contains a specific anti-hLH antibody labeled with a direct label, the specific anti-hLH antibody being freely mobile within the carrier matrix when in the moist state and wherein the capture filament contains an unlabeled specific anti-hLH antibody which is permanently immobilized and is therefore not mobile in the moist state, the labeled and unlabeled anti-hLH antibodies having specificities for different hLH epitopes, the relative positioning of the reagent filament and the capture filament being such that the urine test sample applied to the test device can mobilize the labeled anti-hLH antibody contained in the reagent filament and thereafter permeate to the capture filament where the presence of anti-hLH antibody bound hLH is captured by the capture filament and the moisture impervious hollow casing having at least one aperture through which a detectable response can be observed together with a cap to cover the carrier matrix protruding from the hollow casing.

33. A process for the manufacture of a test device according to claim 1 comprising; cutting a length of carrier matrix, applying onto the carrier matrix a labeled analyte binding reagent, drying the labeled analyte binding reagent onto the carrier matrix, affixing a length of capture filament onto the carrier matrix, inserting the carrier matrix into a hollow casing having at least one aperture through which a detectable response can be observed such that the carrier matrix protrudes from one end of the hollow casing, and affixing a cap onto the hollow casing to cover the carrier matrix protruding therefrom.

34. A process for the manufacture of a test device according to any of claims 2, 31, and 32 comprising; cutting a length of carrier matrix, affixing a length of reagent filament onto the carrier matrix, affixing a length of capture filament onto the carrier matrix wherein the relative positioning of the reagent filament and the capture filament being such that the test sample applied to the test device can mobilize the labeled binding reagent contained in the reagent filament and thereafter permeate to the capture filament, inserting the carrier matrix into a hollow casing having at least one aperture through which a detectable response can be observed such that the carrier matrix protrudes from one end of the hollow casing, and affixing a cap onto the hollow casing to cover the carrier matrix protruding therefrom.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05748

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :G01N 33/543
US CL :436/518

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : Please See Extra Sheet.

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- Y	US 5,079,142 A (COLEMAN et al.) 07 January 1992, see entire document.	1, 3, 5, 6, 9, 10, 12, 14, 16, 18-20 ---------- 26-27
Y	US 4,943,522 A (EISINGER et al.) 24 July 1990, see entire document.	1-30,33,34
Y	US 4,515,889 A (KLOSE et al.) 07 May 1985, see entire document.	1-30,33,34

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 17 JULY 1997	Date of mailing of the international search report 04 AUG 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer CHRISTOPHER CHIN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05748

Box I ***Observations where certain claims were found unsearchable*** **(Continuation of item 1 of first sheet)**

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such *an extent that no meaningful international search can be carried out, specifically:*

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ *No required additional search fees were timely paid by the applicant. Consequently, this international search report is* restricted to the invention first mentioned in the claims; it is covered by claims Nos.:
1-30, 33, and 34

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US97/05748

B. FIELDS SEARCHED
Minimum documentation searched
Classification System: U.S.

422/55-58;
435/6, 287.1, 287.2, 287.7, 287.9, 805, 810, 970;
436/169, 514, 518, 528, 805, 810, 817